

# TOXICOLOGICAL AND BIOCHEMICAL EFFECTS OF SOME RECOMMENDED AND ALTERNATIVE COMPOUNDS ON COTTON LEAFWORM *Spodoptera littoralis* (BOISD) (LEPIDOPTERA:NOCTUIDE) IN COTTON FIELDS.

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## ABSTRACT

Field and laboratory experiments were carried out to evaluate the two insect growth (IGRs), lufenuron and teflubenzuron, antifeedant compound indoxacarb, mineral oil (Kz oil), compound Protecto, *Bacillus thuringiensis* (Bt) and Dursban, chlorpyrifos against the larvae of cotton leafworm, *Spodoptera littoralis* (Boisd.). Field experiment conducted during 2013 and 2014 seasons at Kafr Sakr region, Sharkia Governorate, Egypt. Results revealed that chlorpyrifos recorded highest initial reduction (89.38 and 88.39%), residual mean (88.52 and 87.72%) and annual mean (87.74 and 87.37%) on *Spodoptera littoralis* during the two successive seasons, respectively.

In regarding to the biochemical activities of treated larvae in laboratory the all tested compounds disrupted the tested activities. The highest effect on the total soluble protein as specific activity (SA) of 40.57 mg/g bwt recorded for 4<sup>th</sup> instar larvae treated with LC<sub>50</sub> concentration of chlorpyrifos and sampled after 3 days of treatment, while the highest reduction in relative activity (RA%) of -54.15% recorded for larvae treated with LC<sub>50</sub> concentration of Betavant and sampled after 3 days also. The tested compounds at selected concentrations of LC<sub>25</sub> and LC<sub>50</sub> also disrupted GOT and GPT activities of treated larvae where the highest GOT as SA (2574.33±30.4 u \* 10<sup>3</sup> g bwt) recorded for larvae treated with LC<sub>25</sub> concentration and sampled after 3 days of treatment, while the highest relative activity RA% -43.36% was exhibited in case of after 7 days of treatment. The larvae treated with chlorpyrifos and sampled. The highest effect on GPT as SA (711.33±5.2 u \* 10<sup>3</sup> g bwt) was recorded for larvae treated with Komatch at concentration of LC<sub>25</sub> and sampled at 3<sup>rd</sup> days of treatment, while the highest relative activity% -64.05 recorded for larvae treated with LC<sub>50</sub> concentration of Betavant and sampled at 3<sup>rd</sup> day of treatment also. In regarding to the effect of tested compounds on carbohydrate hydrolyzing enzymes; invertase, trehalase and amylase determined as µg glucose/min g bwt/days. The highest values were 405.679±9.03, 222.33±7.26 and 123±3.31 recorded for larvae treated with LC<sub>25</sub> of Kz oil, LC<sub>50</sub> of Kz oil and LC<sub>25</sub> of Betavant sampled at 3<sup>rd</sup> day of treatment for invertase, trehalase and amylase enzymes, respectively. On the other hand, the highest relative activity RA% of -0.53, -0.44 and -0.61 were recorded for larvae treated with LC<sub>50</sub> of Nomult (at 3 days of treatment), LC<sub>25</sub> of Nomult (at 3<sup>rd</sup> day of treatment) and LC<sub>50</sub> of Kz oil (at 7 days of treatment) that for invertase, trehalase and amylase, respectively.

**Keywords:** *Spodoptera littoralis* (Boisd.), Toxicity, Biochemical, IGRs, indoxacarb, mineral oil, *Bacillus thuringiensis* (Bt).

## INTRODUCTION

In Egypt, Cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Nectuidae) is a serious lepidopteran pest of cotton through its different growth stages, where the larvae are heavily attacking cotton causing severe damage and consequently reduction in the obtained yield. (Pluschkellet *et al.*, 1998 and Korrat *et al.*, 2012). To control cotton leafworm, many compounds use from different pesticides groups, biopesticides, oils and plant extracts. The antifeedant compound, indoxacarb (Betavant 5% EC) was effected the newly ecdysed 2<sup>nd</sup> and 4<sup>th</sup> instars larvae of *S. littoralis* and the LC<sub>50</sub>, LC<sub>90</sub> values, were 0.63 and 3.1 ppm for the 2<sup>nd</sup> instar and 2.0 & 18.75 ppm for the 4<sup>th</sup> instar larvae. That mean the 2<sup>nd</sup> instar larvae was more susceptible to indoxacarb more than the 4<sup>th</sup> one (Al-Shannaf *et al.* 2012). Al-Shannaf and Ammar (2011) stated that, the Radical (Avermectin) compound gave highest initial reduction percentage against *S. littoralis* and *Helicoverpa armigera* followed by Dursban (chlorpyrifos), mixture of Consult (IGR) and Dursban only, where the lowest reduction percentage was recorded for Dipel DF (Bt). As results of Mohamed *et al.* (2006), the mineral oil Kapl-2 at rate of 1.5 and 0.75% showed low effect on *S. littoralis* in comparison by the insecticide, Actellic (pirimiphos methyl). In the same trend, the mineral oil, Kemesol 95% used as

topical application reduced hemolymph fat body and total soluble protein of *S. littoralis*, (Khatter and Abuldaib, 2010). The IGR compound, teflubenzuron affected GOT enzymes activities and total soluble protein for *S. littoralis* larvae significantly, while its effect on GPT, was a significant reduction, (EL-Kordy *et al.*, 1995) and Desuky, *et al.*, 2005). The insecticide Betavant (Indoxacarb) caused slightly increasing in total protein content of *S. littoralis* 2<sup>nd</sup> instar larvae by 8.79%, while it decreased total soluble protein by 24.9% in 4<sup>th</sup> instar and disrupted carbohydrate enzymes, as results of Gmail *et al.* (2011). Also, the bacterial insecticide *B. thuringiensis* and Kz oil reduced the total protein content of treated *S. littoralis* (Zidan *et al.*, 1996).

This work aimed to study the toxic (as field trials) and physiological (as laboratory trials) effects of some recommended and alternative compounds, i.e., Komatch, Nomult, Betavant, Kz oil, Protecto and Dursban against cotton leafworm, *S. littoralis*.

## MATERIALS AND METHODS

### Tested Compound:

#### 1. Insect growth regulators (IGRs):

- Komatch, Lufenuron 5% EC used at rate of 160 cm/ feddan

- Nomult, Teflubenzuron 5% EC used at rate of 160 cm/feddan.
- 2. Antifeedant compound: Betavant, indoxacarb 14.3% SC used at rate of 110cm/feddan.
- 3. Meniraloil, Kz oil 95% EC used at rate of 1500 ml/100 litter water.
- 4. Bacterial Compound: Protecto, *Bacillus thuringiensis* 9.4% WP at rate of 300gm/feddan
- 5. Organophosphorus insecticide: Dursban, chlorpyrifos 48% E.C. used at rate 1000 ml/ Feddan.

**Field trials:**

Field experiments were carried out at Kafr Sak region, Sharkia Governorate, Egypt during two consecutive cotton growing seasons of 2013 and 2014. The experiment area of two faddan was divided into 6 treatments and one as control (and each replicated three times). The experiment area was cultivated with the Egyptian cotton variety, Giza 86. Cotton plants treated once with each compound at 24<sup>th</sup> and 21<sup>th</sup> June during the considered seasons,

respectively. The samples of 100 plants/ replicate were inspected in field and 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> instars larvae were counted and recorded, Just before treatment and after 1, 7 and 10 days for Dursban treatment, while it was examined at 3, 7 and 10 days for each of Komatch, Nomult, Betavant, Kzoil and Protecto. The reduction percentages of cotton leafworm larvae were calculated using the equation of Henderson and Tilton (1955).

**1-Insect Rearing:**

Cotton leafworm, *Spodoptera littoralis* larvae were obtained from a culture reared in cotton leafworm laboratory at Plant Protection Research Institute Sharkia branch without exposure history to insecticide. Larvae were reared on fresh castor bean leaves, *Ricinus communis* L. All laboratory trials were kept under laboratory condition of 27±2C° and RH% 70±5%.

**2. Laboratory treatment:**

10 individuals The cotton leafworm 4<sup>th</sup> instar larvae were put in glass Jar, replicated 4 times for each treatment and labeled as treatments and control.

**Table (1): LC<sub>50</sub> and LC<sub>25</sub> values of the tested compounds.**

Compound	Komatch*	Nomult*	Betavant*	Kzoil*	Protecto**	Dursban*	Control*
LC <sub>25</sub>	2.52	1.17	0.16	5521.01	82.24	6.66	0.00
LC <sub>50</sub>	12.08	15.89	1.41	27734.36	303.42	31.24	0.00

\* Concentration in ppm \*\* Concentration as international unit

Table (1) cleared that, the LC<sub>25</sub> and LC<sub>50</sub> concentrations were prepared as water solution and the castor bean leaves were cleaned and dipped in each separately. The dipped castor bean leaves were left for 30 min. to complete dryness on table under laboratory conditions. After that, the treated leaves of each concentration of each compound delivered to the 4<sup>th</sup> instar larvae in glass jars as well as the leaves dipped in water only as control. The larvae were fed on treated castor leaves for three days for all treatment except of Dursban which fed for 24 h. only, then the all fed on untreated leaves till the end of experiment.

**3. Samples preparation:**

The 4<sup>th</sup> instar larvae samples were collected at 3; 7 days post treatment with LC<sub>25</sub> and LC<sub>50</sub> concentrations of each tested compound as well as untreated one. Samples were homogenized in distilled water using a Teflon homogenizer, the homogenates were centrifuged at 5000 rpm for 10 min. at 5°C the supernatants were immediately assayed to determine the total soluble protein, the activities of aspartate amino transferase (Got) and a alanine amino transferase (GPT) and the carbohydrate hydrolyzing enzymes (Trehalase, Invertase and Amylase).

**4. Determination of biochemical activities:**

**a- Carbohydrate hydrolyzing enzymes:**

The method used to determine the activities of carbohydrate hydrolyzing enzymes (Trehalase, Invertase and Amylase) digesting sucrose, Trehalase and starch, respectively, were illustrated by Ishaaya and Swirski (1976). The free aldehydes group of glucose after starch, Trehalase and sucrose digestion was determined using 3, 5 dinitro salicylic acid reagent.

**b- Determination of total soluble protein:**

Colorimetric determination of (TSP) total homogenized *S. littoralis* larvae was carried out as described by Bradford, M.M. (1976). Protein reagent was prepared by dissolving 100mg of Coomassie Brilliant blue G-250 in 50ml 85% (W/V) Phosphoric acid were added. The resulting solution was diluted to a final volume of 1 liter.

**c- Transaminase enzymes determination:**

Aspartate amino transferase (GOT) and alanine amino transferase (GPT) enzyme activities were determined calorimetrically according to method of (Reitmin and Frankal 1957).

**Statistical analysis:**

One way ANOVA was used to determine the significance of differences between, means of values obtained in the field experiment.

**RESULTS AND DISCUSSION**

**1-Field evaluation of tested compounds on cotton leafworm:**

The obtained results in Table (2) showed that the initial effect as reduction percentages of the cotton leafworm larvae in fields sprayed with recommended concentration of the tested compound at 2013 season were 81.71, 83.61, 41.18, 7.53 and 11.09% after 3 days of spray with lufenuron, teflubenzuron, indoxacarb, Kzoil and BT respectively, while it was 89.53% after one day for chlorpyrifos. As residual effect, the reduction percentages were 87.66, 85.07, 45.94, 8.54 and 13.52% after 7 days and were 88.93, 86.78, 53.00, 14.21 and 18.29% after 10 days for lufenuron, teflubenzuron, indoxacarb, Kz oil and *B.thuringiensis*, respectively,

recorded 87.66 and 86.38% for chlorpyrifos after 7 and 10 days of application, respectively. The insecticide chlorpyrifos recorded the highest reduction percentage of 87.74% while Kz oil recorded the lowest one 8.87%.

**Table (2): Reduction percentage of some alternative compounds against cotton leafworm, *Spodoptera littoralis* compared with Dursban in cotton fields during 2013 season.**

Treatments	Pre-count	Initial		Residual		Mean	Annual mean
		1day	3days	7days	10days		
Komatch	No.	121.25	-	17.93	17.08	22.56	19.82
	%	-	-	88.93	86.17	81.71	8.94
Nomolt	No.	147.61	-	20.20	22.78	25.05	23.92
	%	-	-	86.78	85.07	83.61	83.84
Betavant No.	No.	143.25	-	68.19	78.70	85.34	82.02
	%	-	-	53.00	45.94	41.18	43.66
KZ oil	No.	141.28	-	128.83	132.22	133.22	132.72
	%	-	-	14.21	8.54	7.53	8.04
Protecto	No.	136.97	-	113.75	120.53	123.78	122.16
	%	-	-	18.29	13.52	11.09	12.32
Dursban	No.	165.81	17.78	-	20.91	22.83	19.35
	%	-	89.38	-	87.66	86.53	88.52
Control	No.	153.97	151.77	156.70	159.61	162.89	161.25
F. test							**
LSD <sub>0.05</sub>							6.17

During 2014 season, data in Table (3) showed that the initial effect as reduction percentages of cotton leafworm larvae after 3 days of field spray were 83.48, 82.11, 43.44, 7.42 and 11.17% for lufenuron, teflubezuron, indoxacarb, Kz oil and *B.thuringiensis*, respectively, while it was 88.39% for chlorpyrifos after one day of spray. The residual effect recorded after 7 and 10 days of application cleared that the chlorpyrifos recorded highest reduction percentage of

87.06 & 88.36% followed descendingly by 84.91, 83.76, 46.97, 8.16 & 13.49% at 7 days and 83.48, 82.11, 43.44, 11.17 & 4.2% at 10 days for lufenuron, teflubezuron, indoxacarb, *B.thuringiensis* and Kz oil, respectively; in the field population of the cotton leafworm larvae in sprayed fields. These results found in agreement with those of Mohamed *et al.* (2006); AL-Shannaf and Ammar (2011) and Barrania (2013).

**Table (3): Reduction percentage of some alternative compounds against cotton leafworm, *Spodoptera littoralis* compared with Dursban in cotton fields during 2014 season.**

Treatments	Pre-count	Initial		Residual		Mean	Annual mean
		1 day	3 days	7 days	10 days		
Komatch	No.	147.66	-	19.09	22.67	24.73	23.70
	Reduction %	-	-	86.75	84.91	83.48	84.49
Nomolt	No.	125.81	-	29.49	30.79	33.88	32.34
	Reduction %	-	-	84.45	83.76	82.11	82.94
Betavant	No.	-	-	83.86	93.88	97.80	95.84
	Reduction %	-	-	52.62	46.97	43.44	45.21
KZ oil	No.	174.11	-	162.17	171.46	172.83	172.15
	Reduction %	-	-	13.13	8.16	7.42	7.79
Protecto	No.	183.65	-	159.51	165.87	169.79	167.83
	Reduction %	-	-	16.65	13.49	11.17	12.34
Dursban	No.	188.17	19.73	-	23.32	24.64	21.53
	Reduction %	176.59	88.39	-	87.06	86.34	87.72
Control	No.	186.77	187.97	189.42	191.80	193.81	192.91
F. test							**
LSD <sub>0.05</sub>							6.16

The statistical analysis results cleared that there are significant differences between Dursban, Komatch and Nomult as high efficacy compounds, Betavant as

moderate efficacy compound and the lowest efficacy group, Kz oil and Bt compound, that as initial effect (LSD= 6.16) and as residual effect after 7 days

(LSD=7.37).The same trend was noticed for general mean and residual mean with LSD = 6.46 and 6.16,respectively.

**2-Laboratory trials: Biochemical responses of cotton leafworm, *S. littoralis* larvae to the tested compound:**

The physiological changes of *S. littoralis* 4<sup>th</sup> instar larvae assessed at 3 and 7 days after treatment with LC<sub>25</sub> and LC<sub>50</sub> concentrations of the tested compounds were determined as, effects on the activities of carbohydrate hydrolyzing enzymes (Invertase, Trehalase and amylase) ,the total soluble protein concentration and transaminase( GOTandGPT) were determined.

**a- carbohydrate hydrolyzing enzymes activities:**

Data presented in table (4) indicated that, the all tested compounds were effected the activities of amylase, Invertase and Trehalase enzymes in the treated 4<sup>th</sup> instar larvae of *S. littoralis*.

**• Invertase**

In case of the effect of LC<sub>25</sub> concentration of tested compound on invertase activity in treated and untreated 4<sup>th</sup> instar larvae sampled after 3 days of treatment as SA and RA% in treated larvae in relation to untreated ones. The highest effect on Invertase was recorded in larvae treated with Kz oil (405.67±9.03 µ g glucose / min / g. bwt days) recorded increase in RA% of 0.25% in compared with untreated larvae while the lowest SA was recorded in larvae treated with Nomult (204±6.91 µg glucose / min / g. bwt days) recorded reduction of -0.37% in compared with (325±5.209 µg glucose / min / g. bwt days) for untreated larvae.

In the same trend, the result of LC<sub>50</sub> treatment effect after 3 days cleared that the highest SA was recorded in larvae treated with Kz oil (365.67±6.39 µg glucose / min / g.bwt days) recorded relative increase of 0.13% in compared with untreated larvae while the lowest was recorded with Nomult (154.67±2.23 µg glucose / min / g.bwt) recorded reduction of -0.53% in compared with 325±12.04 µg glucose / min / g. bwt days) for untreated larvae.

**Table (4): Changes in invertase,trehalase and amylase enzymes activities in *S. littoralis* treated with multiple compounds and chemical pesticide**

Treatments	Con. ppm	Invertase (Ug glucose/min/g.bwt/days		Trehalase Ug glucose/min/g.bwt		Amylase Ug glucose/min/g.bwt	
		3 days	7 days	3 days	7 days	3 days	7 days
Komatch	25 SA	260.00±3.78	270.67±4.34	121.33±4.54	212.33±6.24	88.33±2.23	91.67±4.18
	RA %	-0.20	0.09	-0.28	-0.07	0.11	-0.06
	50 SA	212.00±1.42	247±4.05	129.67±4.75	201.33±6.37	46.33±1.52	103.67±0.88
	RA %	-0.35	-0.01	-0.23	-0.12	-0.42	0.07
Nomolt	25 SA	204.00±6.91	236.33±3.18	95.67±3.14	243.33±3.39	82.00±2.63	115.33±3.72
	RA %	-0.37	-0.05	-0.44	0.07	0.03	0.19
	50 SA	154.67±2.23	244.00±3.61	72.67±1.79	251.67±5.79	57.33±3.20	104.67±3.85
	RA %	-0.53	-0.02	-8.57	0.10	-0.28	0.08
Betavant	25 SA	312.67±4.96	256.33±4.85	181±3.10	204.33±7.18	123.00±3.31	100.67±3.39
	RA %	-0.04	1.33	0.07	-0.11	0.54	0.04
	50 SA	310.67±4.39.	250.00±5.52	155.33±1.66	218.00±4.73	102.67±2.33	92.67±1.77
	RA %	-0.05	0.01	-0.001	-0.05	0.28	-0.05
KZoil	25 SA	405.67±9.03	149.33±15.19	208.00±6.08	145.33±3.49	89.00±1.25	77.00±4.05
	RA %	0.25	-0.40	0.23	-0.37	0.11	-0.21
	50 SA	365.67±6.39	147.33±7.43	222.33±7.26	112.33±6.24	105.33±3.58	38.00±2.52
	RA %	0.13	-0.41	0.32	-0.51	0.32	-0.61
Protecto	25 SA	279.67±7.65	236.00±6.04	166.33±2.89	210.00±1.53	58.33±2.69	105.67±3.18
	RA %	-0.14	-0.06	-0.02	-0.08	-0.27	0.09
	50 SA	242.33±10.17	253.00±7.52	104.00±3.46	247.33±3.85	36.00±2.50	91.33±1.86
	RA %	-0.26	0.01	-0.39	-0.08	-0.55	-0.06
Dursban	25 SA	246.33±5.63	179.67±1.86	149.00±9.44	279.67±7.23	92.00±1.441	64.33±2.73
	RA %	-0.24	-0.20	-0.12	0.22	0.15	-0.34
	50 SA	215.00±6.39	189.67±6.07	154.67±6.55	258.67±4.10	93.00±3.88	43.00±3.52
	RA %	-0.34	-0.24	-0.09	0.13	0.16	-0.56
Control		325.00±12.09	249.67±11.85	169.00±5.72	222.67±12.72	80.00±4.33	97.00±4.71

SA= Specific activity  
 RA%= Relative concentration  
 $RA\% = \frac{\text{Treatment} - \text{control}}{\text{Control}} \times 100$

The effect of LC<sub>25</sub> concentration of tested compound on Invertase activity in treated and untreated 4<sup>th</sup> instar larvae sampled after 7 days after treatment as SA and RA% in treated larvae in relation to untreated ones. The highest AS was recorded in larvae treated with

Komatch (270.67±4.34 µg glucose / min / g.bwt days) recorded increase in RA% by 0.09% in compared with untreated larvae. While the lowest SA was recorded in larvae treated with Kz oil (149.33±15.19 µg glucose/ min/ g.bwt days) recorded reduction of -0.40% in

compared with  $249.67 \pm 11.85$   $\mu\text{g}$  glucose / min / g.bwt days for untreated larvae.

In case of  $\text{LC}_{50}$  treatment after 7 days, The highest effect was recorded on larvae treated by Betavant ( $250 \pm 5.52$   $\mu\text{g}$  glucose min g.bwt days) recorded increase in RA% by 0.01% in compared with untreated ones, while the lowest effect was recorded with Kz Oil ( $147.33 \pm 7.43$   $\mu\text{g}$  glucose / min / g.bwt days) recorded reduction of -0.41% in compared with  $249.67 \pm 11.85$   $\mu\text{g}$  glucose / min / g.bwt days for untreated larvae.

The results above found agree those of Ahmed, *et al.* (1990); Kandil, (2005); EL-Kordy, *et al.* (1995); and Gamil, *et al.* (2011). recorded that the Kz oil effected invertase activity in *S. littoralis*.

#### • Trehalase

Data in table (4) showed the effect of  $\text{LC}_{25}$  concentration of tested compound on trehalase activity in treated and untreated 4<sup>th</sup> instar larvae after 3 days as SA and RA% in treated larvae in relation to untreated ones. The highest effect on trehalase was recorded in larvae treated with Kz oil ( $208 \pm 6.02$   $\mu\text{g}$  glucose / min / g.bwt days) recorded increase in RA% by 0.23% in compared with untreated larvae, while, the lowest SA was recorded in larvae treated with Nomult ( $95.67 \pm 3.14$   $\mu\text{g}$  glucose / min / g.bwt days) recorded increase in RA% by 0.44% in compared with  $169 \pm 5.72$   $\mu\text{g}$  glucose / min / g. bwt days for untreated larvae.

In the same trend, the results of  $\text{LC}_{50}$  treatment after 3 days cleared that the highest effect on Trehalase was recorded in larvae treated with Kz oil ( $169 \pm 5.72$   $\mu\text{g}$  glucose / min / g bwt days) recorded increase in RA% by 0.32% compared with untreated larvae, while the lowest effect was recorded in larvae treated use Nomult ( $72.67 \pm 1.79$   $\mu\text{g}$  glucose / min / g. bwt days ) recorded reduction of -8.57% compared with for untreated larvae

The effect of  $\text{LC}_{25}$  concentration of tested compound on Trehalase activity in treated and untreated 4<sup>th</sup> instar larvae sampled after 7 days of treatment as SA and RA% in treated larvae compared with untreated ones. The highest SA was recorded in larvae treated with Dursban ( $279.07 \pm 7.23$   $\mu\text{g}$  glucose/ min / g. bwt days) recorded increase in RA% by 0.22% in compared with untreated larvae, while the lowest effect was recorded in larvae treated with Kz oil ( $145.33 \pm 3.49$   $\mu\text{g}$  glucose / min / g. bwt days) recorded reduction of -0.37% in compared with ( $222.57 \pm 12.72$   $\mu\text{g}$  glucose / min / g bwt days ) for untreated larvae .

The results of  $\text{LC}_{50}$  treatment after 7 days revealed that the highest SA was recorded in larvae treated with Dursban ( $258.57 \pm 5.09$   $\mu\text{g}$  glucose/ min / g. bwt days) recorded increase in RA% by 0.13% compared with untreated larvae, while the lowest SA was recorded in larvae treated with Kz oil ( $112.33 \pm 6.24$   $\mu\text{g}$  glucose / min / g. bwt days) recorded reduction of -0.51% in compared with ( $222.57 \pm 12.72$   $\mu\text{g}$  glucose/ min/ g. bwt days) for untreated larvae

The results of these trial found in agreement with those of Ayyangar and Rao (1990); Kandill (2000); Desuky, *et al.* (2005); Omar *et al.* (2005) and (Sabry and

Khedr, 2014) .The IGRs and chlorpyrifoseeffected Trehalase activity in *S. littoralis*.

#### • Amylase

Data in table (4) showed the effect of  $\text{LC}_{25}$  concentration of tested compound on amylase activity in treated and untreated 4<sup>th</sup> instar larvae after 3 days of treatment as SA and RA% in treated larvae in relation to untreated ones. The highest effect on amylase was recorded in larvae treated with Betavant ( $123 \pm 3.31$   $\mu\text{g}$  glucose / min / g. bwt days) recorded increase in RA% by 0.54% in compared with untreated larvae, while the lowest SA was recorded in larvae treated with Protecto ( $58.33 \pm 2.69$   $\mu\text{g}$  glucose / min / g. bwt days) recorded reduction of -27% in compared with ( $80.0 \pm 4.33$   $\mu\text{g}$  glucose/ min/ g. bwt days) for untreated larvae.

The results of  $\text{LC}_{50}$  treatment after 3 days showed that The highest SA was recorded in larvae treated with Kz oil ( $105.33 \pm 3.58$   $\mu\text{g}$  glucose / min / g. bwt days) recorded increase in RA% by 0.32% in compared with untreated larvae, while the lowest SA was recorded in larvae treated with Komatch ( $46.33 \pm 1.52$   $\mu\text{g}$  glucose/ min / g. bwt days ) recorded reduction of -0.42% in compared with ( $80.0 \pm 4.33$   $\mu\text{g}$  glucose / min / g. bwt days) for untreated larvae

In regarding to the effect of  $\text{LC}_{25}$  concentration of tested compound on amylase activity in treated and untreated 4<sup>th</sup> instar larvae tested at 7 days after treatment as SA and RA% in treated larvae in relation to untreated ones. the highest SA on amylase was recorded in larvae treated with Nomult ( $115.3380.0 \pm 4.333.72$   $\mu\text{g}$  glucose / min / g. bwt days) recorded increase in RA% by 0.19% in compared with untreated larvae, while the lowest SA was recorded for larvae treated with Dursban ( $64.3380.0 \pm 4.333.43$   $\mu\text{g}$  glucose/ min/ g. bwt days) recorded reduction of -0.34% in compared with  $97.0080.0 \pm 4.334.64$   $\mu\text{g}$  glucose / min / g. bwt days for untreated larvae .

In case of the results of  $\text{LC}_{50}$  treatment after 7 days, the highest SA was recorded in larvae treated with Nomult ( $104.67 \pm 3.80$   $\mu\text{g}$  glucose/ min/ g. bwt days) recorded increase in RA% 0.08% in compared with untreated larvae, while the lowest effect was recorded in larvae treated with Kz oil ( $38 \pm 2.52$   $\mu\text{g}$  glucose/ min/ g. bwt days) recorded reduction of -0.61% in compared with  $97.00 \pm 4.94$   $\mu\text{g}$  glucose / min / g. bwt days) for untreated larvae.

These results found in agreement with those of Wyatt (1957); Wiggles worth (1972); Nathon, *et al.* (2005) and EL-Sheikh, *et al.* (2013) stated that, the tested IGRs and Kz oil effected Amylase activity in *S. littoralis*.

#### b -Total soluble protein (TSP) assessment:

Data in Table (5) showed the effect of  $\text{LC}_{25}$  concentration of tested compound on TSP levels in treated and untreated 4<sup>th</sup> instar larvae sampled after 3 days as specific concentration (SC) and relative concentration (RC%) in treated larvae in relation to untreated ones. The highest effect on TSP was recorded in larvae treated with Dursban ( $38.60 \pm 0.26$  mg / g.bwt) recorded reduction of -0.18% in compared with untreated larvae while the lowest effect was recorded in

larvae treated with Betavant (21.20±0.56 mg/g.bwt) recorded increase of 45.18% in compared with 38.67mg/g.bwt for untreated larvae.

In the same trend, the results of sampled larvae treated by LC<sub>50</sub> after 3 days cleared that the highest effect on TSP was recorded in larvae treated with Dursban (40.57±0.59mg/g. bwt) recorded increase of 5.17% in compared with untreated larvae while the lowest effect was recorded in larvae treated with Betavant (17.73±0.38mg/g. bwt) recorded increase of 54.15% in compared with 38.57±0.26mg/g.bwt for untreated larvae.

In case of the effect of LC<sub>25</sub> concentration of the tested compound on TSP levels in treated and untreated 4<sup>th</sup> instar larvae sampled after 7 days as SC and RC% in treated larvae in relation to untreated ones. The highest effect was recorded in larvae treated with Protecto (32.63±0.70mg/g. bwt) recorded increase of 4.82% in

compared with untreated larvae, while the lowest effect was recorded in larvae treated with Kz oil (23.87±0.65mg/g.bwt) recorded increase of 23.32% in compared with 31.13mg/g.bwt) for untreated larvae. In the same trend the results of the effect on larvae treated with LC<sub>50</sub> treatment after 7 days cleared that, the highest effect on TSP was recorded in larvae treated with Protecto (33.37±0.54 mg/g.bwt) recorded increase of 7.20% in compared with untreated larvae while the lowest effect was recorded in larvae treated with Nomult (18.90±0.38mg/g.bwt) recorded increase of 39.29% in compared with 31.13±1.56 mg/g.bwt for untreated larvae that after 7 days of treatment with LC<sub>50</sub> concentration. These results found agree those of EL-Kordy et al.(1995); Zidan et al.(1996); Desuky, et al.(2005); Gamilet al.(2011) and Basiouny, et al.(2016) that tested compounds effected TSP in *S. littoralis* and other insects.

**Table (5): Changes of total soluble protein levels in *S. littoralis* treated with multiple compounds and chemical pesticide**

Treatments	Days post treatment	Determined parameter	Komatch		Nomolt		Betavant		Kzoil		Protecto		Dursban		control
			25	50	25	50	25	50	25	50	25	50	25	50	
Total proteins (mg/g. bwt)/days	3 days	SC	31.53 ± 0.59	23.03 ± 0.58	34.17 ± 0.38	31.20 ± 0.39	21.20 ± 0.56	17.73 ± 0.38	35.90 ± 0.80	36.30 ± 0.74	25.77 ± 0.55	25.57 ± 0.24	38.60 ± 0.26	40.57 ± 0.59	38.67 ± 0.85
		RC%	-18.46	-30.10	-11.64	-19.32	-45.18	-54.15	-7.16	-6.13	-33.36	-33.88	-0.18	5.17	
	7 days	SC	24.10 ± 0.20	24.70 ± 0.61	25.83 ± 0.38	18.90 ± 0.55	26.07 ± 0.87	20.57 ± 0.65	23.87 ± 0.65	20.77 ± 0.65	32.63 ± 0.70	33.37 ± 0.54	30.33 ± 0.38	20.13 ± 1.21	31.13 ± 1.56
		RC%	-22.58	-20.66	-17.03	-39.29	-16.26	-33.92	-23.32	-33.33	4.82	7.20	3.13	-35.34	

SC = Specific concentration  
 RC% = Relative concentration  
 $RC \% = \frac{\text{Treatment} - \text{control}}{\text{Control}} \times 100$

**c- Transaminase activities:**

Data in table (5) showed the changes in GOT, GPT activities as the concentration of the formed pyruvate and the relative activity as a percentage of formed pyruvate for treated larvae in comparable with untreated ones. The obtained results cleared that the all tested compound affected the transaminase activities positively or negatively as follows:

• **The effect on Aspartate amino transferase (GOT):**

Data in table (6) showed the effect of LC<sub>25</sub> concentration of tested compound on GOT activity in treated and untreated 4<sup>th</sup> instar larvae sampled after 3 days of treatment as specific activity (SA) and relative activities (RA%) in treated larvae in relation to untreated ones. The highest effect was recorded in larvae treated with Kz oil (2574.33±30.41u\* 10<sup>3</sup> g.bwt) recorded increase of 0.33% in compared with untreated larvae while the lowest effect was recorded in larvae treated with Betavant (1441.67±24.56 u\* 10<sup>3</sup> /g.bwt) recorded reduction of (-26.36%) in compared with (1957.67±29.19 u\* 10<sup>3</sup> g.bwt) for untreated larvae.

In the same trend the results of the affected treated larvae with LC<sub>50</sub> treatment after 3 days cleared that, the highest effect was inspected in larvae treated with Kz oil (2305±7.88 u\* 10<sup>3</sup> g.bwt) recorded increase of 31.50% in compared with untreated larvae, while the lowest effect was recorded in larvae treated with

Betavant (1266.67±4.25 u\* 10<sup>3</sup> g.bwt) recorded reduction of -35.30% in compared with (1957.67±29.19 u \* 10<sup>3</sup> g.bwt) for untreated.

In regarding to the effect of LC<sub>25</sub> concentration of the tested compound on GOT activities in treated and untreated 4<sup>th</sup> instar larvae sampled at 7 days after treatment as SA and RA% in treated larvae in relation to untreated ones. The highest SA was recorded in larvae treated with Komatch (1615 ±9.88 u \* 10<sup>3</sup> g. bwt) recorded increase in RA% by 9.86% in compared with untreated larvae, while the lowest effect was recorded in larvae treated with Dursban (832.57 ±19.40 u \* 10<sup>3</sup> g. bwt) recorded reduction of -43.36% compared with (1470 ±65.65 u\* 10<sup>3</sup> g.bwt) for untreated larvae.

In the same trend the results of LC<sub>50</sub> treatment after 7 days cleared that, the highest SA was recorded in larvae treated with Komatch (1509±5.14 u \* 10<sup>3</sup> g.bwt) recorded increase in RA% by 2.65% in compared with untreated larvae, while the lowest effect was recorded in larvae treated with Dursban (601.00±9.55 u\* 10<sup>3</sup> g.bwt) recorded reduction of -59.12% in compared with (1470±65.65 u\* 10<sup>3</sup> g.bwt) for untreated larvae.

The obtained results found in agreement with those of Ahmed, et al. (1990); EL-Kordy, et al. (1995); Zidan, et al. (1995); Kandil (2005) and Omar, et al. (2005) that the mineral oil affected GOT activities in *S. Littoralis*

**Table (6):**Changes of total soluble protein levels, GOT and GPT activities in *S. littoralis* treated with multiple compounds and chemical pesticide.

Treatments	Con. ppm		GOT (Ux 10 <sup>3</sup> g. bwt)		GPT (Ux 10 <sup>3</sup> g. bwt)	
			3 days	7 days	3 days	7 days
Komatch	25	SA	1951.00±11.73	1615.00±9.88	711.33±5.20	502.33±7.23
		RA %	-0.34	9.86	16.42	9.84
	50	SA	2044.67±22.65	1509.00±5.14	667.33±12.79	454.67±7.37
		RA %	0.05	2.65	9.22	-0.58
Nomolt	25	SA	1937.00±27.04	1511.67±22.45	587.33±33.15	404.67±5.18
		RA %	-1.06	2.84	-3.87	-11.52
	50	SA	1900.67±20.90	1482.00±10.52	637.33±14.63	322.67±4.66
		RA %	-2.91	0.82	4.31	-29.45
Betavant	25	SA	1441.67±24.56	980.00±30.59	397.67±2.77	395.00±7.27
		RA %	-26.36	-33.33	-34.92	-13.63
	50	SA	1266.67±4.25	1046.33±27.54	219.67±7.85	305.67±5.18
		RA %	-35.30	-28.82	-64.05	-33.16
KZoil	25	SA	2574.33±30.41	1553.33±19.19	384.00±13.91	256.67±12.19
		RA %	0.32	5.67	-36.66	-43.88
	50	SA	2305.00±7.88	1489.67±9.18	241.00±12.11	221.67±11.48
		RA %	31.50	1.16	-60.06	-51.53
Protecto	25	SA	2002.67±11.07	1526.67±16.73	636.33±7.01	457.67±8.46
		RA %	0.18	3.39	4.15	0.08
	50	SA	1863.33±56.90	1461.67±17.07	699.33±4.26	537±18.79
		RA %	-4.82	-0.06	14.46	17.42
Dursban	25	SA	1821.00±10.16	832.67±19.40	323.67±5.59	238.67±8.18
		RA %	-6.98	-43.36	-61.92	-47.81
	50	SA	1917.67±23.38	601.00±9.55	303.67±7.01	259.33±10.99
		RA %	-7.15	-59.12	-50.30	-43.30
Control			1957.67±29.19	1470.00±65.65	611.00±13.29	457.33±23.98

SA= Specific activity

RA%=  $\frac{\text{Relative concentration Treatment} - \text{control}}{\text{Control}} \times 100$

RA% =  $\frac{\text{Relative concentration Treatment} - \text{control}}{\text{Control}} \times 100$

• **-Alanine amino transferase (GPT)**

Data in table (6) showed the effect of LC<sub>25</sub> concentration of tested compound on GPT activity in treated and untreated 4<sup>th</sup> instar larvae sampled after 3 days of treatment as specific activity (SA) and relative activity (RA%) in relation to untreated ones. The highest effect on GPT was recorded in larvae treated with Komatch (711.33±5.20 u \* 10<sup>3</sup>g.bwt) recorded increase of 16.42% in compared with untreated larvae, while the lowest SA was recorded in larvae treated with Dursban (323.60±5.39 u \* 10<sup>3</sup>g.bwt) recorded reduction of -61.92% in compared with 611.00±13.29 u \* 10<sup>3</sup> g. bwt) for untreated larvae.

In the same trend, the results of LC<sub>50</sub> treatment after 3 days cleared that, the highest SA was recorded in larvae treated with Protecto (699.33±4.25 u \* 10<sup>3</sup> g.bwt) recorded increase of 14.46% in compared with untreated larvae, while the lowest AS was recorded for Betavant (219.67±7.83 u \* 10<sup>3</sup> g.bwt) recorded reduction of -64.05% in compared with (611±13.29 u \* 10<sup>3</sup> g.bwt) SA for untreated larvae.

In regarding to the effect of LC<sub>25</sub> concentration of tested compound on GPT activity in treated and untreated 4<sup>th</sup> instar larvae sampled after 7 days as SA and RA% in treated larvae in relation to untreated ones. The highest AS was recorded in larvae treated with Komatch (502.33±7.23 u \* 10<sup>3</sup> g.bwt) recorded

increase in RA% by 9.84% in compared with untreated larvae, while the lowest effect was recorded for larvae treated with Dursban (238.67± 8.18 u \* 10<sup>3</sup> g.bwt) recorded reduction of -47.81% in compared with (457.23±23.98 u \* 10<sup>3</sup> g.bwt) for untreated larvae.

In the same trend the results of LC<sub>50</sub> concentration after 7 days cleared that, the highest SA recorded in larvae treated with Protecto (537±18.79 u \* 10<sup>3</sup> g.bwt) recorded increase of 17.42% in compared with untreated larvae, while the lowest effect was recorded in larvae treated with Kz oil (221.67±11.48 u \* 10<sup>3</sup> g.bwt) recorded reduction of -51.53% in compared with (457.33±23.98 u \* 10<sup>3</sup> g.bwt) for untreated larvae. These results relatively in affinity with those of EL-Kordy, *et al.* (1995); Kandil (2005); Abou-Taleb, *et al.* (2015); and Hamadah, *et al.* (2016). The mineral oil effected GPT activity in *S. littoralis*.

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## التأثيرات السامة والبيوكيميائية لبعض المركبات البديلة والموصى بها على دودة ورق القطن (Noctuide:Lepidoptera) (*Spodoptera littoralis* (Boisd.) في حقول القطن.

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أجريت التجارب الحقلية والمعملية لتقييم إثنين من منظمات النمو الحشرية (ليفينرون والتفلوبنزيرون) ومركب مانع للتغذية (اندوكسكارب) والزيت المعدني (كزداول) والمركب البكتيري (باسيلس ثيروجينيز) والمركب الفوسفوري العضوي (كلوربيريفوس) وذلك ضد يرقات دودة ورق القطن .

وأجريت التجارب الحقلية خلال موسمي ٢٠١٣ - ٢٠١٤ في منطقة كفر صقر - بمحافظة الشرقية - مصر، وأوضحت النتائج أن مركب الكلوربيريفوس أعطى أعلى خفض فوري (٨٩.٣٨ ، ٨٨.٣٩%) وكذلك متوسط أثر متبقى (٨٨.٥٢ ، ٨٧.٧٢%) ومتوسط عام (٨٧.٧٤ ، ٨٧.٣٧%) على دودة ورق القطن خلال موسمي الدراسة على الترتيب.

وبالنظر إلى نتائج المعاملات المعملية والمتمثلة في النشاط البيوكيميائي لليرقات المعاملة، فإن جميع المركبات المختبرة سببت اضطرابات في الأنشطة البيوكيميائية. سجل أعلى تأثير على تركيز البروتينات الكلية (٤٠.٥٧ مللي جرام / جرام وزن الجسم ليرقات العمر الرابع المعاملة بالتركيز LC<sub>50</sub> من مركب الكلوربيريفوس وذلك بعد ٣ أيام من المعاملة بينما سجلت أعلى نسبة خفض في تركيز البروتينات الكلية (- ٥٤.٠١٥%) لليرقات المعاملة بمركبة الإندوكسكارب بعد ٣ أيام أيضاً مقارنة باليرقات الغير معاملة.

كما أدت المعاملة بالتركيزات المختبرة (LC<sub>25</sub>) و (LC<sub>50</sub>) لجميع المركبات اضطرابات في نشاط الإنزيمات الناقلة لمجموعة الأمين في اليرقات المعاملة حيث سجل أعلى تأثير على GOT (٣٣.٣٣.٥٧٤ وحدة دولية في ١٠<sup>٢</sup> من وزن الجسم) لليرقات المعاملة بتركيز LC<sub>25</sub> للزيت المعدني وذلك بعد ثلاث ايام من المعاملة بينما سجلت أعلى خفض في النشاط النسبي (- ٤٣.٣٦%) لليرقات المعاملة بنفس التركيز لمركب الكلوربيريفوس وذلك بعد ٧ أيام من المعاملة.

سجل أعلى تأثير على نشاط GPT (٥.٢ ± ١١١.٣٣ وحدة دولية ١٠<sup>٣</sup> جرام وزن جسم) لليرقات المعاملة بمركب الليفينرون وبالتركيز LC<sub>25</sub> وذلك بعد ثلاث أيام من المعاملة ، بينما سجلت أعلى نسبة خفض للنشاط النسبي (- ٦٤.٠٥%) لليرقات المعاملة بتركيز LC<sub>50</sub> من مركب الإندوكسكارب وذلك بعد ثلاث أيام من المعاملة أيضاً مقارنة باليرقات الغير معاملة.

وبخصوص تأثير المركبات المختبرة على الإنزيمات المحللة للكربوهيدرات كانت ٩.٠٣ ± ٥٥.٦٧٩ ، ٧.٢٦ ± ٢٢٢.٣٣ ، ٣.٣١ ± ١٢٣.٠٠ سجلت لليرقات المعاملة بالزيت المعدني (تركيز LC<sub>50</sub> والاندوكسكارب (تركيز LC<sub>25</sub>) وذلك بعد ثلاث أيام من المعاملة ، على الترتيب. وإختلف النشاط النسبي عن ذلك حيث سجل أعلى خفض للنشاط النسبي - ٥٣.٠٠ ، - ٤٤.٠٠ ، - ٦١.٠٠% لليرقات المعاملة بتركيز LC<sub>50</sub> لمركب التيفلوبنزورون وذلك بعد ثلاث أيام والتركيز LC<sub>25</sub> لنفس المركب بعد ثلاث أيام من المعاملة أيضاً وكانت الأخيرة لليرقات المعاملة بالزيت المعدني بتركيز LC<sub>50</sub> بعد سبعة أيام وذلك للثلاث أنزيمات على الترتيب. وذلك للمقارنة لليرقات الغير معاملة.